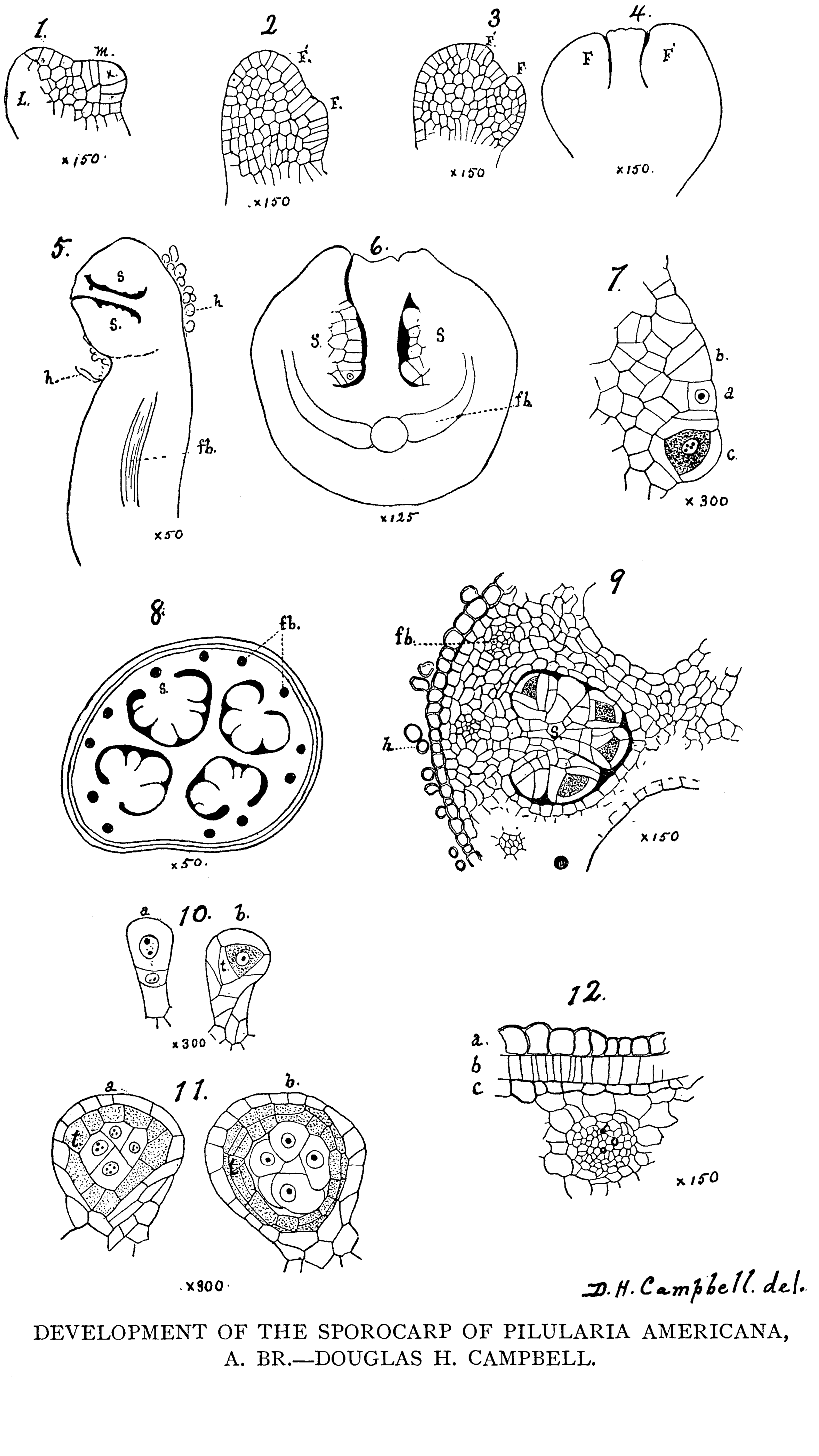

DEVELOPMENT OF THE SPOROCARP OF PILULARIA AMERICANA, A. BR.—DOUGLAS H. CAMPBELL.

The Development of the Sporocarp of Pilularia Americana, A. Br.

BY DOUGLAS HOUGHTON CAMPBELL.

(PLATE CXLVI.)

Pilularia Americana is by no means uncommon in California, and is said to be especially abundant in the Sacramento Valley. Last spring a quantity of fresh specimens, collected near Suisun, were sent me by Mrs. Brandegee, and as they bore numerous sporocarps, in all stages of develoyment, it seemed a good opportunity to attempt to clear up some obscure points in the early development of the sporocarps of the Marsiliaceæ.

Without microtome sections, it is quite impossible to study the earliest stages satisfactorily on account of their small size, and because they are completely buried in a mass of hairs that cover the growing point of the stem, and all of the younger parts in its vicinity. Those hairs cannot be removed from the young sporocarp and interfere very seriously with a clear view of it.

Our knowledge of the development of the sporocarp of *Pilularia* is based mainly upon the work of Sachs,* Goebel,† and Juranyi,‡ and that of *Marsilia* upon Russow's investigations.§ The origin of the sporocarp itself, however, and the formation of the cavities in which the sporangia are borne, was not clearly understood.

By imbedding in paraffine in the usual way and sectioning with a microtome, no trouble was experienced in getting a complete series of preparations that showed all the details, and the results of a study of these is here given. In preparing the specimens for sectioning, the growing tips of the fruiting plants were treated for two or three hours with a 1 per cent. aqueous solution of chromic acid, washed, stained *in toto* with alum cochineal, and after sectioning, stained on the slide with alcoholic Bismarck-brown; my usual method of preparing sections of young tissues.

* Sachs, Text-Book of Botany, 2 ed., pp. 455–460.

† Goebel, "Outlines," p. 240, foot note.

‡ Jurányi. The original paper in Hungarian. Reported in Just's Jahresbericht, Jahrgang 1879, p. 416.

§ Russow. Histologie und Entwickelungsgeschichte der Sporenfrucht von Marilia. Dorpat, 1871.

The creeping stem of *Pilularia* grows from a single tetrahedral apical cell, from which three series of segments are cut off, as in most ferns. Two of these series give rise to the two rows of leaves, and from the third, roots only are formed. The stem branches freely, the branches arising close behind its apex.

Leaves of two kinds are formed, fertile and sterile; the former differ from the latter simply in bearing a sporocarp. The foliar nature of the sporocarp has been inferred from analogy with the ferns, and also from a study of the position of the older sporocarps but the way in which it originates from the leaf was not known. Juranyi * states that in *P. globulifera*, the formation of the fruit begins, only after the leaf has reached a considerable size. My own observations on *P. Americana* do not confirm this. On the contrary, the yonng fruit begins to develop almost as soon as the leaf can be recognized, and while it is still close to the apex of the stem, long before it is large enough to be seen with the naked eye.

The young leaf, like the stem, grows from a tetrahedral apical cell. Growth at first is stronger upon the outer side, and in consequence, even at a very early stage, the leaf is strongly coiled, as in all the homosporous Leptosporangiates. In the fertile leaves, however, before this curvature has become very pronounced, a protuberance may be noticed upon its inner face, not far above the base. (Fig. 1, M.) This originates from the growth of a single cell (x), which acts as an apical cell in the same way as that of the apex of the body of the leaf. This protuberance is the young sporocarp which at this stage is clearly seen to be simply a segment or branch of the fertile leaf.

The young sporocarp enlarges rapidly after its formation and assumes the form of a blunt cone. Next, on the side turned toward the sterile segment of the leaf which bears it, a slight prominence is noticed (Fig. 2, F.), and about the same time two similar lateral prominences are formed. As in the sterile segment growth is stronger on one side (here the side turned away from the sterile segment), and in consequence the sporocarp bends over toward the sterile segment of the leaf. The apex of the young sporocarp (Fig. 2, F.), together with the three prominences referred to, enclose a slightly depressed area which is top of the young sporo-

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* L. c.

carp. The four prominences (including the original apex of the fertile segment), are beyond doubt to be regarded as leaflets, which, however, are never much elevated above the surface of the young fruit, and in this first stage form four slightly elevated ridges.

A little later (Fig. 3), these become more prominent, and a slight depression or pit is formed between the base of each and the cells occupying the top of the young sporocarp. These pits are separated laterally by the coherent margins of the leaflets which extend to the axis of the sporocarp and are continuous with it. The young fruit now enlarges rapidly, and as it does so the depressions deepen owing to the elongation of the leaflets, and also to that of the cells of the axis of the sporocarp, which form a sort of columella running through the center.

The leaflets, or as we will now call them, lobes, are only free at their tips; and as the edges are in contact from the first and extend to the axis, the clefts between them and the axis form four deep cavities that open by as many pores at points opposite the tips of the lobes. These pits correspond to the "canals" described by Russow in the fruit of Marsilia.

Juranyi states that these cavities are caused by a splitting apart of the cells of the inner tissue of the sporocarp, and that the communication with the outside is brought about by the subsequent separation of the cells at the apex of the sporocarp; that is, that the cavities are endogenous in origin. Doubt has been expressed as to the accuracy of these statements, and Goebel states that his observations do not bear them out.

A study of longitudinal sections of the young sporocarp show beyond doubt the strictly external origin of these cavities.

Up to the time that the cavities begin to form, the young fruit is composed of a uniform, small-celled parenchyma, but a little later, however, the primary tissue systems are differentiated, and the separation of the body of the sporocarp from its peduncle becomes evident. About the same time the axial cells in the basal part of the sporocarp extending into the peduncle, elongate and form the beginning of the single fibro-vascular bundle that traverses the peduncle and joins that of the sterile segment of the leaf near its base.

The peduncle grows rapidly and becomes several times longer than the sporocarp itself—(Fig 5). The growth upon the upper side of the latter is stronger than upon the lower side, and in consequnce, it becomes bent over, nearly or quite at right angles to the peduncle. With the enlargement of the sporocarp the cavities within become deeper and wider in a direction parallel to the broad surfaces of the lobes; but the radial growth of these keeps pace with the longitudinal growth, so that the space between the inner surface of the lobe and the columella, is very narrow. The growth is especially active in the inner epidermal cells, which project more or less and form a cushion running vertically along the median line of each lobe. This cushion is the sorus (Fig. 5. 6. 8.), and as its surface cells develop into the sporangia, it nearly fills the cavity in which it lies. A tranverse section at this time shows that the portions separating the cavities are composed of about four layers of cells.

The fibro-vascular bundle which traverses the center of the peduncle divides into four branches at the base of the sporocarp, and one of these goes to each lobe and forms a sort of midrib (Fig. 6.). Later each of these bundles sends off two lateral branches that follow the margins of the lobe. A cross section of a sporocarp at this stage (see Fig. 9), shows these as groups of small cells at nearly equal distances from each other, one at the center of the lobe, the others close to its margin. By this time the epidermal cells of the outside of the sporocarp begin to thicken. This is the first indication of the hard shell found in the ripe fruit.

While these changes are going on in the outer tissues of the sporocarp, the sporangia have begun to develop from the surface cells of the sori. Active growth begins in these cells, which become elevated as papillæ above the surface of the sorus. This is most marked in the basal or older cells, but proceeds rapidly toward the upper end of the sorus. While in a general way we may say that the sporangia arise in acropetal succession, still new ones arise later among the earlier formed ones, without reference to their position; indeed all of the surface cells of the sori may be regarded as potentially, at least, sporangium mother-cells.

There is a good deal of variation in *Pilularia*, as in many ferns, as to the direction of the first division walls in the young sporan-

gium, and it seems to me that too much stress has been laid upon this by some writers.

In the earlier sporangia the first division walls are usually strongly oblique, but even here the first wall may be transverse (see Fig. 7, a.), as usually happens in the Polypodiaceæ. Several oblique walls now arise which meet each other in such a way that the terminal cell has much the form of an ordinary tetrahedral apical cell. Soon, however, a wall is formed parallel to the outer wall of the terminal cell, and thus an inner cell (Fig. 7, c.), the archesporium, is cut off. The archesporium is formed relatively earlier in the first formed sporangia which are almost sessile, while in the later ones, where they are more crowded, the pedicel is much better developed (Fig. 10).

From the archesporium the tapetal cells (t.), are cut off in the usual way, and subsequently these divide by both radial and tangential walls into a large number of cells. For the most part there is but one set of tangential divisions, but sometimes there is a second one in some of the cells, so that at these points the tapetum is three cells thick.

The central cell divides by an oblique wall into two cells and these each divide twice more, and sometimes some or all of the resulting cells may divide again, so that the whole number of the resulting spore mother-cells ranges from eight to sixteen.

When the full number is complete the cells separate owing to the partial disintegration of the division walls, and, at the same time, the walls of the tapetal cells become completely absorbed and their contents form a mass of protoplasm in which the separated spore mother-cells are imbedded. These now increase somewhat in size and become globular as the pressure of the surrounding cells is removed. The sporangium has now increased a good deal in size and the spore mother-cells do not completely fill it.

In fresh sporangia the tapetal cells appear completely disorganized, but when stained sections are examined it is evident enough that the protoplasm and nuclei of the cells persist unchanged, and the further development shows that the protoplasm and nuclei of the tapetum probably play an important part in the further growth of the spores.

The division of each of the spore mother-cells into four shows nothing peculiar. The nucleus divides twice before any division of the protoplasm takes place, and the four daughter-nuclei arrange themselves at equal distances from each other near the periphery of the cell, after which division walls are formed simultaneously between them, and the resulting spores are of the tetrahedral type.

Up to this point the sporangia are all alike, but now a difference is noticed between those in the lower and those in the upper part of the sporocarp. The former develops into macrosporangia, the latter into microsporangia. In the latter all of the young spores come to maturity, but in the former one spore very early begins to grow faster than the others, which finally shrivel up and develop no further. The young macrospore is at first nearly globular, but soon becomes oval, and finally completely fills the sporangium. In its early stages the membrane is thin, but as it grows it becomes very thick. A slight examination shows that the spore is surrounded by a layer of protoplasm, in which are imbedded a great many nuclei. This protoplasm is no doubt that derived from the tapetum, and its position indicates that it, with its included nuclei, is actively concerned in the nourishment of the growing macrospore. This office is probably two-fold; first to provide material or the growth of the spore contents, and secondly to deposit upon the outside of the spore the material for the formation of the peculiar and highly specialized spore coat, characteristic of the Marsiliaceæ. The development of the spore membrane seems to be the same in the microspores, but owing to their smaller size is not so easy to trace out. The wall of the sporangium remains but one cell thick, and shows no traces of the annulus found in all the terrestrial leptosporangiate ferns. This complete disappearance is in allprobability to be traced to a loss of function. In the terrestrial ferns, the opening of the sporangium is brought about by drying up, and the contraction of the annulus by drying is the principal factor in the process. In the Marsiliaceæ, on the contrary, the sporangium only opens when its walls are dissolved by the action of water. Possibly further search will show some trace of an annulus in the earlier stages of the sporangium, but I could discover none, either in the young or ripe sporangia.

As the sporocarp ripens the outer cells become very hard, especially the first layer of hypodermal cells (Fig. 12, b.), whose walls become finally so much thickened that the cell cavities are almost completely obliterated. The second hypodermal layer also has its walls more or less thickened, but not nearly to the same extent.

The ripe sporocarp is about 3 mm. in diameter, and the peduncle about as long and bent downward, so that the sporocarp is partially or completely buried in the earth. When perfectly ripe it splits into four valves corresponding to the lobes or leaflets of which it is made up. This splitting follows the median line of the partitions in the sporocarp.

A comparison of the foregoing statements with the corresponding points in the development of *Marsilia*, so far as the latter is known, show, as might be expected, close resemblances. There seems no doubt that the sporocarp is simply a portion of a leaf, bearing much the same relation to the sterile part that the fertile pinnæ of *Ophioglossum* or *Osmunda* for example, do the sterile part of the leaf. We may perhaps more aptly compare it to such a fern as Onoclea, which is really more nearly related. The structure of one of the spore-bearing leaf segments of *O. sensibilis* for instance, is very similar, indeed, to the sporocarp of *Pilularia*, except that the sporangia are borne upon the lower and not upon the upper side of the leaf. As the Marsiliaceæ are in all probability descendants of forms related to the Polypodiaceæ, the origin of these peculiar points is probably to be looked for in forms having fertile leaves of a type similar to *Onoclea*.

On comparing *Pilularia Americana* with *P. globulifera*, of Europe, less difference was found than is generally supposed.* Except the longer peduncle of the fruit of the former and a slightly diminished development of the wall of the macrospore, I could see no difference. In size my specimens were little, if any, inferior to specimens of *P. globulifera* studied by me in Europe, either as regards the leaves or the sporocarp; and, almost without exception, the sporocarp was four-chambered as in that species, instead of three-chambered as described in the text-books. The absence of

* Goebel, L. c., p. 240. Underwood, "Our Native Ferns and Their Allies," 3d. ed., pp. 126–127. Watson, "Botany of California," Vol. ii., p. 352.

a well marked constriction in the macrospore is due simply to a slightly diminished development of the outer layers of the spore-wall in the upper part of the spore, and a trace of this can always be found in the older spores. At any rate the two species must be regarded as very closely related.

Explanation of Plate CXLVI.

The magnification is indicated before each figure.

Fig. 1. The base of a very young fertile leaf of *Pilularia Americana*, showing the beginning of the sporocarp, m. its apical cell.

Fig. 2. Longitudinal section of an older sporocarp—F. F. two of t e young lobes.

Fig. 3. A similar section of a somewhat older sporocarp.

Fig. 4. A still older sporocarp, in which the cavities are well developed.

Fig. 5. Longitudinal section of on older sporocarp, including the peduncle. S. S. sori; H. hairs; fb. fibro-vascular bundle of the peduncle.

Fig. 6. Longitudinal section of about the same age as in Fig. 5, but at right angles to the peduncle.

Fig. 7. Longitudinal section of the young sorus, a. b. c. young sporangia.

Fig. 8. Transverse section of an older sporocarp, showing the four cavities.

Fig. 9. A single cavity from a somewhat younger one.

Figs. 10, 11. Young sporangia, t. the tapetum.

Fig. 12. Part of the wall of a nearly full grown sporocarp, a. b. c. the outer thick-walled cells.

Free Nitrogen Assimilation by Plants.

By H. W. Conn.

The study of bacteriology has introduced to us an entirely new realm of knowledge. Twenty-five years ago the scientific world had little conception of the great change that was to be made in our knowledge of the processes of nature by the development of the study of micro-organisms. That bacteria were the cause of certain diseases was even then strongly believed by many and had been definitely proved in a few cases. But that micro-organisms, in general, lay at the foundation of many of the most important physiological processes of nature was not even dreamed of. The difficulty of research in this line made it possible for only a very